THE MUCIN OF WHITE FIBROUS CONNECTIVE TISSUE

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R. H. CHITTENDEN

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WILLIAM J. GIES

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By R. H. CHITTENDEN, Ph. D.,
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WILLIAM J. GIES, Ph. B.

(*Contributions from the Sheffield Biological Laboratory of Yale University.*)

All of the bodies belonging to the group of mucins and mucoids are possessed of considerable physiological interest, owing especially to their peculiar compound nature and the illustration which they afford of a possible intimate union between the proteid group and carbohydrate radicles. That there are a number, possibly a large number, of closely related bodies belonging to the mucins and mucoids there can be no question. Thanks to the labours of Hammarsten * and his pupils, many of these bodies have been subjected to careful and thorough investigation, and much light has been thrown upon their relationships and differences. There is still, however, much to be ascertained regarding these bodies, and any additional facts broadening or substantiating our present knowledge are to be welcomed as contributing toward a more complete understanding of their genetic relationships. The union of carbohydrate groups with proteid molecules is probably more common than has hitherto been supposed, as witness the peculiar gluco-nucleoproteid recently described by Hammarsten † as a constituent of the pancreas and other glands, and the identification by Kossel ‡ of a peculiar carbohydrate group as a cleavage product of certain forms of nucleic acid. Presumably in these compound proteids of the mucin type the character of the proteid radicle as well as of the carbohydrate radicle is subject to variation, and it is easy to conceive of differences in the nature and properties of the mucins dependent upon varia-

* Pflüger's *Archiv f. Physiol.*, Band xxxvi; *Zeitschr. f. physiol. Chem.*, Band x and xii.
† *Zeitschr. f. physiol. Chem.*, Band xix.
‡ Du Bois-Reymond's *Archiv f. Physiol.*, Physiol. Abtheil., 1891.

tions in the amount and character of both the carbohydrate and proteid groups. The ready formation of acid-albumin, or syntonin, albumoses and peptone when mucins are decomposed by the action of superheated water or boiling dilute acids, affords ample evidence of the presence of true proteid radicles in the bodies of this class, although we do not know definitely the exact nature of the proteid groups present in the original molecule. On the other hand, the simultaneous formation of reducing bodies whenever mucins are broken down by the action of dilute acids, and the separation of a dextrin-like body (the animal gum of Landwehr *) by cleavage with superheated water, clearly indicate the existence of some form of carbohydrate matter in the mucin molecule.

Of the true mucins present in the tissues of the higher animals, the mucin of the submaxillary gland and the corresponding body present in or between the fibres of ordinary connective tissue are the most important from a physiological standpoint. The former is a product of the metabolic activity of secretory cells which are among the most active of the secreting cells of the body, while the latter is a product of a tissue whose activity is certainly of a low order. That these two mucins, though closely related, are unlike, is clearly indicated by their divergence in chemical composition as well as by their general reactions and properties.

Loebisch,† whose careful study of the mucin from tendons constitutes the chief source of our knowledge regarding the chemical composition of this body, ascribes to tendon-mucin the formula $C_{160}H_{256}N_{32}S_1O_{80}$, with a molecular weight of 3,936. Such a formula calls for the presence of 0·81 per cent of sulphur, and this amount was found by Loebisch in the three preparations of mucin from ox tendons analyzed by him. In a recent examination of mucin prepared from this same source we have obtained quite different results as regards the content of sulphur, and this fact has led us to make a careful study of the composition of this form of connective-tissue

* *Zeitschr. f. physiol. Chem.*, Band viii and ix. Also Pflüger's *Archiv f. Physiol.*, Band xxxix and xl.

† *Zeitschr. f. physiol. Chem.*, Band x, p. 40.

mucin. Our results in the main have afforded a close substantiation of the conclusions arrived at by Loebisch, with the single exception of the sulphur for which we can find no adequate explanation. Further, some additional facts have been found which are perhaps worthy of note.

The first sample of mucin studied was prepared from the Achilles tendons of oxen by the following method, analogous to the method described by Loebisch: The fresh tendons were freed as carefully as possible from all adherent tissues, then cut into very thin transverse sections with a razor, washed thoroughly with distilled water, frequently renewed for twenty-four hours, in order to remove all blood and soluble albuminous matter, and finally pressed as dry as possible. The resultant material weighed 1,200 grammes. In order to extract the mucin, the tissue was placed in 2·4 litres of half-saturated lime-water, where it was allowed to remain for forty-eight hours with frequent agitation. At the end of this period the pale-yellowish fluid was strained through a cloth filter and finally filtered through paper. The clear fluid was then treated with an excess of 0·2-per-cent hydrochloric acid—a little more than a litre—by which a heavy flocculent precipitate resulted, quickly settling to the bottom of the cylinder, leaving a nearly clear supernatant fluid.

The residue of tendon-tissue was again extracted for forty-eight hours with 2·4 litres of half-saturated limewater, and the resultant solution precipitated with an excess of 0·2-per-cent hydrochloric acid. The precipitate so formed was nearly as heavy as the first, thus showing that extraction of the mucin by weak limewater is a slow and gradual process.

The precipitated mucin, separated from the acid fluid by subsidence and decantation of the supernatant liquid, was washed thoroughly with 0·2-per-cent hydrochloric acid, whipping up the precipitate with the fluid and then allowing it to subside, this operation being repeated with fresh quantities of acid until the latter failed to give any proteid reaction. In this manner it was hoped to remove all adherent albuminous matter extracted from the tissue by the limewater. The two portions of mucin were then united and washed

by decantation with distilled water until the acid was entirely removed. As the fluid became less and less acid, more time was required for the precipitate to settle, as the latter tended to swell in the water and was more inclined to float on the surface of the fluid.

The mucin was next dissolved in half-saturated limewater, of which a large volume was required, the solution filtered through paper, and the mucin reprecipitated by the addition of an excess of 0·2-per-cent hydrochloric acid, a small quantity of stronger hydrochloric acid being likewise added to induce a good flocculent separation of the substance. The precipitate was again washed by decantation with 0·2-per-cent hydrochloric acid, and lastly with water, until the acid was entirely removed. Whenever it was necessary for the precipitate to stand for some time with water, the mixture was kept as cool as possible, and a little alcoholic solution of thymol added to guard against putrefactive changes. When the acid was wholly removed from the precipitate the water was replaced by weak alcohol, and finally by ninety-five-per-cent alcohol, repeatedly renewed, until the substance was thoroughly dehydrated, after which the precipitate was collected on a filter and allowed to drain. It was then boiled with alcohol-ether (a mixture of equal parts absolute alcohol and ether) in a suitable flask connected with an inverted Liebig's condenser for many days—i. e., with renewed quantities of alcohol-ether until the latter gave no residue on evaporation. As Loebisch has shown, this is quite an important part of the process of purification, since a certain amount of foreign extractive matter adheres tenaciously to the mucin, and can be removed only by long-continued extraction with the above mixture. When this process was completed the mucin was thrown upon a filter, washed thoroughly with ether, and finally dried over sulphuric acid. When quite dry it presented the appearance of a perfectly white powder, light and fluffy. The yield amounted to twelve grammes of the dry product, and, assuming that the entire amount of mucin had been extracted from the tendons, and disregarding the loss incidental to purification, this quantity would imply the presence in the fresh tendons of one per cent of mucin.

The composition of the product, dried at 110° C. until of constant weight, was as follows: *

PREPARATION No. 1.

I. 0·2670 gramme of substance gave 0·4781 gramme of CO_2 = 48·84 per cent C, and 0·1585 gramme of H_2O = 6·60 per cent H.

II. 0·2277 gramme of substance gave 0·4082 gramme of CO_2 = 48·89 per cent C, and 0·1329 gramme of H_2O = 6·48 per cent H.

III. 0·1975 gramme of substance gave 0·3548 gramme of CO_2 = 48·99 per cent C.

IV. 0·2363 gramme of substance gave 0·1417 gramme of H_2O = 6·66 per cent H.

V. 0·2426 gramme of substance gave, by the Kjeldahl method, 0·02865 gramme of nitrogen = 11·81 per cent N.

VI. 0·2754 gramme of substance gave, by the Kjeldahl method, 0·03246 gramme of nitrogen = 11·79 per cent N.

VII. 0·2784 gramme of substance gave, by the absolute method, 27·63 c. c. of nitrogen at 13·3° C, and 764·7 mm. pressure = 11·96 per cent N.

VIII. 0·3345 gramme of substance gave, by the absolute method, 33·3 c. c. of nitrogen at 13·2° C, and 754·5 mm. pressure = 11·84 per cent N.

IX. 0·5373 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·0943 gramme of $BaSO_4$ = 2·41 per cent S; after deducting sulphur of ash = 2·36 per cent S.

X. 0·4969 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·0856 gramme of $BaSO_4$ = 2·37 per cent S; after deducting sulphur of ash = 2·32 per cent S.

XI. 0·2943 gramme of substance gave 0·0023 gramme of ash = 0·78 per cent ash.

XII. Ash from 0·2943 gramme of substance gave 0·00112 gramme of $BaSO_4$ = 0·05 per cent S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

											Average.
C.	49·22	49·27	49·37	....	.....	.....	.....	.....	....	....	49·29
H.	6·65	6·54	.....	6·71	.....	.....	.....	.....	....	....	6·63
N.	.....	.....	.....	....	11·90	11·88	12·05	11·93	....	....	11·94
S.	.....	.....	.....	....	.....	.....	.....	.....	2·36	2·32	2·34
O.	.....	.....	.....	....	.....	.....	.....	.....	....	....	29·80
											100·00

The second preparation of mucin was made in a somewhat different manner. The fresh tendons, freed as far as possible from foreign tissue, were cut into thin transverse sections, washed with water somewhat, then soaked for thirty-six hours in about four litres of ten-per-cent salt solution, with vigorous agitation from time to time, after which the saline solution was decanted and the tissue washed with water until the chloride was entirely removed. The

* The nitrogen was determined by both the absolute and the Kjeldahl method, while carbon and hydrogen were determined by combustion in oxygen gas in an open tube, the products of combustion passing over a layer of cupric oxide, chromate of lead, and metallic copper.

salt solution on dilution with water gave a distinct turbidity indicating the presence of a globulin. The application of heat likewise produced a precipitate, as did also the addition of dilute acetic and hydrochloric acids. It is thus evident that the salt solution removes at the outset quite an appreciable amount of proteid matter, with perhaps some mucin. The moist tissue, pressed as dry as possible, weighed 1,700 grammes. It was then extracted with 3·4 litres of half-saturated limewater for forty-eight hours, two such extractions being made. From these extracts the mucin was precipitated by the addition of 0·2-per-cent hydrochloric acid, the second extract apparently yielding as heavy a precipitate as the first. The combined precipitates were washed repeatedly by decantation with 0·2-per-cent hydrochloric acid, lastly with water. The mucin was next dissolved in a little 0·5-per-cent sodium carbonate, the solution filtered, made nearly neutral by the addition of a little ten-per-cent hydrochloric acid, so as to avoid undue dilution, and then precipitated by 0·2-per-cent hydrochloric acid. The precipitate was again washed thoroughly with 0·2-per-cent hydrochloric acid, and lastly with water, until the acid was entirely removed. It was then transferred to ninety-five-per-cent alcohol, frequently renewed, and finally boiled with alcohol-ether as long as anything could be extracted. Dried over sulphuric acid, the product came out quite white, but not so bulky as the preceding preparation, and weighed a little over fifteen grammes—an amount equal to about 0·9 per cent of the moist tissue.

As already stated, mucin is not readily extracted from tendons by limewater; at least four cubic centimetres of half-saturated limewater are required for every gramme of tissue in order to insure a complete extraction. Thus, after the second extraction of the above 1,700 grammes of tissue, a third extraction was made, using again three litres of half-saturated limewater. This solution, on treatment with hydrochloric acid, gave a precipitate weighing one to two grammes when purified, but it was noticeable that more acid was required in order to effect a good flocculent separation of the mucin. Even with a fourth extraction of the tissue a little mucin was obtained, showing as a decided turbidity when the alkaline fluid was

made distinctly acid, but it was not until four or five days' standing that a distinct precipitate settled out even on the addition of stronger hydrochloric acid. The amount so obtained, however, was very small.

The composition of the main product obtained from the 1,700 grammes of tissue when dried at 110° C., until of constant weight, was as follows:

PREPARATION NO. 2.

I. 0·3194 gramme of substance gave 0·5659 gramme of CO_2 = 48·32 per cent C, and 0·1815 gramme of H_2O = 6·31 per cent H.

II. 0·4197 gramme of substance gave 0·7471 gramme of CO_2 = 48·54 per cent C, and 0·2446 gramme of H_2O = 6·47 per cent H.

III. 0·4051 gramme of substance gave 0·7189 gramme of CO_2 = 48·39 per cent C, and 0·2353 gramme of H_2O = 6·45 per cent H.

IV. 0·2519 gramme of substance gave, by the Kjeldahl method, 0·02965 gramme of nitrogen = 11·77 per cent N.

V. 0·2578 gramme of substance gave, by the Kjeldahl method, 0·03026 gramme of nitrogen = 11·74 per cent N.

VI. 0·2954 gramme of substance gave, by the Kjeldahl method, 0·03446 gramme of nitrogen = 11·67 per cent N.

VII. 0·6610 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·1131 gramme of $BaSO_4$ = 2·35 per cent S; after deducting sulphur of ash = 2·32 per cent S.

VIII. 0·5248 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·0936 gramme of $BaSO_4$ = 2·45 per cent S; after deducting sulphur of ash = 2·42 per cent S.

IX. 0·6724 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·1140 gramme of $BaSO_4$ = 2·33 per cent S; after deducting sulphur of ash = 2·30 per cent S.

X. 0·3735 gramme of substance gave 0·0025 gramme of ash = 0·67 per cent ash.

XI. Ash from 0·3735 gramme of substance gave 0·00082 gramme of $BaSO_4$ = 0·03 per cent S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average.
C.	48·64	48·87	48·72	.....	.....	.....	....	.....	....	48·74
H.	6·36	6·52	6·50	.....	.....	.....	....	....	....	6·46
N.	.....	.....	.....	11·85	11·82	11·74	....	....	....	11·80
S.	.....	.....	.....	.....	.....	.....	2·32	2·42	2·30	2·35
O.	.....	.....	.....	.....	.....	.....	....	....	....	30·65
										100·00

A third specimen of mucin was prepared as follows: Fifteen hundred grammes of ox tendons were finely divided, the tissue extracted for twenty-four hours with three litres of ten-per-cent salt solution, and then with water until the salt was wholly removed. The tissue was next extracted for sixty hours with three litres of half-saturated limewater. From this solution the mucin could be only partially

separated by the addition of 0·2-per-cent hydrochloric acid, quite a quantity of ten-per-cent acid being required to effect a flocculent precipitation of the substance. This was purified by itself and not subjected to analysis. The tendons were again extracted with three litres of half-saturated limewater for forty-eight hours, and from this solution the mucin was separated as a flocculent precipitate by the addition of 0·2-per-cent hydrochloric acid. This precipitate was purified by washing with 0·2-per-cent hydrochloric-acid, solution in 0·5-per-cent sodium carbonate, reprecipitation with 0·2-per-cent hydrochloric acid, etc., as described under the last preparation. The yield of dry product from this second extraction of the tissue with limewater amounted to 6·5 grammes. Dried at 110° C. until of constant weight, this preparation gave the following results on analysis:

PREPARATION No. 3.

I. 0·3598 gramme of substance gave 0·6292 gramme of $CO_2 = 47·69$ per cent C, and 0·2072 gramme of $H_2O = 6·40$ per cent H.

II. 0·2939 gramme of substance gave 0·5150 gramme of $CO_2 = 47·79$ per cent C, and 0·1725 gramme $H_2O = 6·52$ per cent H.

III. 0·3154 gramme of substance gave 0·5536 gramme of $CO_2 = 47·87$ per cent C.

IV. 0·1644 gramme of substance gave 0·0944 gramme of $H_2O = 6·38$ per cent H.

V. 0·1965 gramme of substance gave, by the Kjeldahl method, 0·02255 gramme of nitrogen = 11·47 per cent N.

VI. 0·2495 gramme of substance gave, by the Kjeldahl method, 0·02825 gramme of nitrogen = 11·32 per cent N.

VII. 0·2574 gramme of substance gave, by the Kjeldahl method, 0.02930 gramme of nitrogen = 11·38 per cent N.

VIII. 0·6046 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·1045 gramme of $BaSO_4 = 2·38$ per cent S; after deducting sulphur of ash = 2·31 per cent S.

IX. 0·5408 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·0931 gramme of $BaSO_4 = 2·37$ per cent S; after deducting sulphur of ash = 2·30 per cent S.

X. 0·3128 gramme of substance gave 0·0031 gramme of ash = 0·99 per cent ash.

XI. Ash from 0·3128 gramme of substance gave 0·00152 gramme of $BaSO_4 = 0·07$ pe cent S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average
C.	48·17	48·26	48·34	....	.....	.....	.....	....	....	48·26
H.	6·46	6·59	.....	6·44	.....	.....	.....	....	....	6·49
N.	.....	.....	.....	....	11·59	11·43	11·50	....	....	11·51
S.	.....	.....	.....	....	.....	.....	.....	2·31	2·30	2·31
O.	.....	.....	.....	....	.....	.....	.....	....	....	31·43
										100·00

A comparison of the composition of these three preparations of

mucin with each other, and with the mucin analyzed by Loebisch and by Hammarsten, brings out certain points of interest which merit attention:

	MUCIN FROM TENDONS.				Snail mucin. Hammarsten.	Submaxillary mucin. Hammarsten.
	Preparation 1.	Preparation 2.	Preparation 3.	Loebisch's average.		
C.....	49·29	48·74	48·26	48·30	50·32	48·84
H.....	6·63	6·46	6·49	6·44	6·84	6·80
N.....	11·94	11·80	11·51	11·75	13·65	12·32
S.....	2·34	2·35	2·31	0·81	1·75	0·84
O.....	29·80	30·65	31·43	32·70	27·44	31·20

Loebisch analyzed three distinct preparations of mucin from ox tendons, in which the carbon, hydrogen, and sulphur showed practically no variation. The nitrogen, however, varied from 11·59 to 11·84 per cent. The average content of nitrogen in his three preparations was 11·75 per cent, identical with the average of our three preparations. It is to be noticed, however, that the carbon of our preparations shows decided variation, and it is also to be observed that a diminution in the percentage of carbon is attended in each case with a diminution in nitrogen. We may suppose that Preparation No. 3 is the purest of our products, and it is seen to agree most closely with the results obtained by Loebisch, except in the content of sulphur. The mucin from the submaxillary gland, as well as the snail mucin, are both characterized by a comparatively high content of nitrogen, while the latter product also shows a higher percentage of carbon.

Our results seemingly justify the assumption that white fibrous connective tissue contains more than one mucin, or else that the mucin obtainable from this tissue is prone to carry with it a certain amount of some other form of proteid matter which the ordinary methods of purification are not wholly adequate to remove. Our experience leads us to the belief that the surest way of obtaining a pure mucin from tendons, or at least one with a low content of carbon and nitrogen, is to first extract the finely divided tissue with ten-per-cent salt solution, then after removal of the salt with water to extract the tissue with half-saturated limewater in the proportion of

two cubic centimetres for every gramme of moist tissue for about twenty-four hours at ordinary room temperature. This extract may be rejected, as it is very liable to yield a mucin with a higher content of nitrogen and carbon. By extracting the tissue a second time with limewater a mucin may be obtained with a lower content of carbon and nitrogen, as in our third preparation. It is purely an assumption, however, to say that this body with its lower percentage of carbon and nitrogen is *pure* mucin. There is at the present time no standard of purity with regard to this body, and it is quite as probable that fibrous connective tissue contains two or more mucins as that there is only one mucin in the tissue, and that any deviation from the figures obtained by Loebisch or by us in Preparation No. 3 is due to the presence of a larger or smaller amount of proteid impurity.

Undoubtedly, preliminary extraction of the tissue with salt solution tends to remove a certain amount of proteid matter, especially globulins, which might otherwise render the product impure, and possibly this is in part the cause of the lower content of carbon and nitrogen in Preparation No. 2 as contrasted with Preparation No. 1. Still there is no certainty on this point, for it is to be remembered that precipitation of the mucin requires the addition of considerable hydrochloric acid beyond neutralization of the alkaline fluid, and this excess of acid would naturally exert a marked solvent action upon any albuminous matter present. That the first limewater extract is liable to yield a mucin with a higher content of both carbon and nitrogen the results fully indicate, and as a direct illustration of the difference in the content of nitrogen in mucin obtained from a first and second extract, we may instance the following experiment: Fifteen hundred grammes of tendons finely divided, as usual, were extracted with ten-per-cent salt solution for two days, then washed with water and placed in three litres of half-saturated limewater for forty-eight hours. This first extract was then strained off, and the tissue treated a second time with a like volume of half-saturated limewater, thus giving a second extract. From the first extract, the mucin was precipitated by hydrochloric acid slightly above 0·2 per cent, the

precipitate washed with 0·2-per-cent hydrochloric acid, then with water, and lastly dissolved in 0·5-per-cent sodium carbonate. From this filtered solution a portion of the mucin was precipitated by addition of 0·2-per-cent hydrochloric acid, while a second portion separated only on addition of a somewhat increased strength of acid. These two fractions were washed thoroughly with 0·2-per-cent acid, then with water, and finally boiled with alcohol-ether until quite free from soluble matter. The yield in the first fraction was 1·4 gramme, and in the second fraction 1·0 gramme. From the second limewater extract the mucin was precipitated with 0·2-per-cent hydrochloric acid, after which it was purified by washing with 0·2-per-cent acid, solution in 0·5-per-cent sodium carbonate, reprecipitation with 0·2-per-cent acid, etc. The content of nitrogen in the three products, when dried at 110° C., was as follows, calculated on the ash-free substance:

FIRST EXTRACT.		SECOND EXTRACT.
First fraction.	Second fraction.	
12·26 N.	11·91 N.	11·51 N.

It is thus seen that the first extraction with limewater furnishes a mucin with a considerably higher percentage of nitrogen than the second extract. It is equally noticeable that the mucin first precipitated—as in the first fraction of the first extract—has a higher percentage of nitrogen than the second fraction, thus indicating that the higher content of nitrogen and probably of carbon also belongs to some body more readily precipitated by acid than the mucin with 11·51 per cent of nitrogen. In view of the great care exercised in all of these preparations, and the ready solubility of ordinary forms of albuminous matter in an excess of hydrochloric acid, especially after they have once been dissolved in an alkaline fluid, we are very much inclined to believe in the existence of several related mucins as components of ordinary white fibrillar connective tissue.

Such a view presents no great difficulty. Submaxillary mucin, for example, differs from tendon mucin by only 0·5 per cent of carbon (48·84 per cent) and about 0·5 per cent of nitrogen (12·32 per cent), although it shows some other points of difference, such as a tend-

ency to undergo alteration by the action of limewater and by being soluble in 0·2-per-cent hydrochloric acid. Indeed, all of the various mucins described show minor points of difference, although agreeing in their general reactions, and it is easy to conceive of the presence of two or more closely related mucins, in tendons, with different elementary composition.

The most remarkable thing, however, connected with the mucins that we have separated from this form of fibrillar connective tissue is the amount of sulphur present in the purified products. In snail mucin, Hammarsten has shown the presence of 1·75 per cent of sulphur, but in the mucin from the submaxillary gland and in the mucin described by Loebisch as contained in tendons, the amount of sulphur has been placed at 0·84 to 0·81 per cent. In all three of our preparations, however, the sulphur present has amounted to at least 2·30 per cent, and, moreover, the agreement in the several products has been very close indeed. The greater portion of this sulphur is closely combined, a small amount only being in the form of the mercaptan group and responding to the reaction with potassium hydroxide and plumbic acetate. We present these figures with some doubt in our own minds, but, having obtained them as the result of most careful work, we see no possible explanation other than that this amount of sulphur is actually present in the mucin molecule. The determinations of sulphur were made after the usual method recommended by Hammarsten—viz., oxidizing the mucin with a mixture of ten grammes NaOH and two grammes KNO_3 in a silver crucible, etc. The sodium hydroxide employed was chemically pure, having been prepared from the metal, and, furthermore, several blank tests were made to prove the freedom of the various chemicals from sulphur. This percentage of sulphur is greater than has ever been accredited to a true mucin, although the mucin from the snail's membrane (mantle-mucin), which is somewhat related to keratin, has been found by Hammarsten to contain a fairly large amount of this element—viz., 1·79 per cent.

With regard to the *reactions* of the several products that we have studied, there is nothing special to be said. They all show the ordi-

nary reactions of mucin as described by Loebisch, and we can simply substantiate what has long been published by him upon this point.

The most characteristic feature of mucin is the peculiar cleavage it undergoes when heated with dilute hydrochloric acid, by which a substance with reducing action upon alkaline copper solution results. Albumose and peptone are likewise formed by the action of the hot acid. We have tried several preliminary experiments in this direction, the results of which may be briefly stated: 3·25 grammes of mucin of Preparation No. 2 were heated in a boiling water-bath with one hundred cubic centimetres of two-per-cent hydrochloric acid for five hours. At the end of this period the solution was of a deep-brown colour, while suspended through the fluid was a large amount of gelatinous matter more or less brown in colour. This was filtered off, washed with water, in which it was wholly insoluble, until the washings gave no proteid reaction. It was then tested with the following results: It was insoluble in dilute and stronger hydrochloric acid, but readily soluble in 0·5-per-cent sodium carbonate and in very dilute (0·5 per cent) potassium hydroxide. From the solution in sodium carbonate, it was reprecipitated by neutralization, and was then readily soluble in a slight excess of 0·2-per-cent hydrochloric acid. It gave the ordinary colour reactions characteristic of proteid matter. Warmed at 40° C. with an active gastric juice containing 0·2-per-cent hydrochloric acid, it was wholly unaffected even after twenty-four hours, but when warmed with an alkaline pancreatic juice it was readily dissolved, and almost completely converted into products soluble even on neutralization of the fluid, thus attesting its conversion into soluble albumoses and peptones. These reactions suggest that the substance in question is a form of anti-albumid.

The original acid fluid containing the soluble products formed in the cleavage of the mucin was made neutral, by which a slight neutralization precipitate resulted, evidently syntonin from the reactions tried. The neutral fluid was then concentrated to a sirup, a strong caramel-like odour being developed during the process, and while still warm the residue was treated with a large excess of

ninety-five-per-cent alcohol, by which a thick gummy mass was formed, hard and brittle on cooling. While warm, the alcoholic fluid was quite clear and yellowish-red in colour, but on cooling, a light-yellow precipitate, very small in quantity, formed, which was soluble in water, and gave a strong reducing action with Fehling's solution. It was too small in quantity, however, to study further. The gummy precipitate was washed by warming it repeatedly with fresh quantities of alcohol. It was readily soluble in water, gave more or less of a proteid reaction, and showed a fairly strong reducing action with Fehling's solution. Tested with phenylhydrazine hydrochloride, and sodium acetate, only an amorphous precipitate resulted from which a crystalline osazone could not be obtained. On boiling the gummy mass with two-per-cent hydrochloric acid, however, and then extracting the neutralized and evaporated fluid with alcohol, a very small amount of a crystalline osazone was obtained by application of the hydrazine test, apparently identical with that described further on.

The original alcoholic solution from the above gummy precipitate was evaporated to a small bulk on the water-bath, the residue taken up with fifteen cubic centimetres of water forming a clear solution. This solution showed strong reducing action with alkaline copper solution, and evidently contained the greater portion of the reducing body formed from the cleavage of the mucin. To the main bulk of this solution was added one gramme of phenylhydrazine hydrochloride and 1·5 grammes of sodium acetate, after which the mixture was heated on the water-bath for an hour and a half, the volume of the fluid being kept at fifteen to twenty cubic centimetres. While hot the fluid was perfectly clear and reddish in colour. After standing an hour in a cool place there was a marked separation of amorphous particles and oily globules, but no crystals could be detected under the microscope. After standing fifteen hours the amorphous particles were almost wholly transformed into fine crystals. These crystals were light yellow in colour, and were mostly arranged in rosettes or balls of fine yellow needles, somewhat resembling lactosazone. The oily globules were unchanged. These

crystals were purified by dissolving them in cold alcohol, followed by the addition of water, and heating the solution until the alcohol was practically all removed, when the crystals again separated out as the fluid cooled. The crystals were also insoluble in the hot *concentrated* fluid. In this way the crystals were gradually freed from the oily globules spoken of above and rendered fairly pure. Each time the crystals were filtered they were also washed with a little cold water. During the process of purification the crystals changed their appearance somewhat, tending to take on the branching form characteristic of dextrosazone. This crystalline osazone, when purified as much as possible, was readily soluble in warm water, in alcohol, ether, chloroform, and, to a certain extent, in benzol. The amount of the purified osazone was so small that the melting point alone could be determined. This was done as usual in a capillary tube. When the temperature reached 140° C. the substance commenced to darken slowly, and at 160° C. it began to melt. Further recrystallization of the osazone did not alter this melting point. In melting point, therefore, this osazone, if pure, differs widely from dextrosazone or lactosazone. In general appearance and solubility, as well as in its melting point, it appears to resemble very closely the osazone obtained by Hammarsten from the cleavage product of the peculiar nucleoproteid described by him as present in the pancreas.* Whether this body is a pentaglucose, however, we can not definitely say. We had hoped, especially in view of the strong reducing action of the above alcoholic solution, to obtain a fairly large amount of an osazone, sufficient to determine its content of carbon and nitrogen, but the yield of purified product was very small indeed.

In order to verify the above results, a second portion of mucin was decomposed with dilute acid—4·75 grammes of mucin with two hundred and fifty cubic centimetres of 2·0-per-cent hydrochloric acid—the mixture being heated directly over a lamp for about five hours. The flask was connected with an inverted Liebig's condenser to prevent concentration, and the mixture was kept in a state of gentle ebullition. In this case there was much less of the antialbumid-like

* *Zeitschr. f. physiol. Chem.*, Band xix, p. 19.

body so prominent in the first decomposition, the amount being less than one fifth that found before. The neutralization precipitate, however, was considerably larger, and albumose and peptone were both present in abundance. The caramel-like body precipitated by alcohol was naturally more abundant than in the first case, but on analysis it was found to contain a large percentage of nitrogen, so that its fancied resemblance to caramel is purely superficial. By evaporation of the alcoholic extract containing the greater portion of the reducing body a residue was obtained as before, from which a crystalline osazone was formed agreeing in all of its properties with the body previously described. The purified osazone melted at 158° to 160° C. It is thus evident that the mucin or mucins present in ox tendons yield on cleavage with dilute hydrochloric acid a carbohydrate body which forms a well-defined and crystalline osazone, although at present we can not state definitely the exact nature of this carbohydrate substance.